REMARKS

Claims 1-7 and 16-22 are pending. Applicants herewith amend claims 1-5, 16, 18 and 19 in order to better clarify the invention. No claims are added or canceled. No new matter has been added with the amendment. Thus, claims 1-7, 16 and 18-22 are active in this application. Applicants note the Examiner's recognition that these claims are allowable over the prior art of record.

In the Office Action at page 2, the Examiner states that he did not receive the abstract submitted with the Preliminary Amendment of August 27, 1992. In response, applicants herewith attach another copy of the submitted abstract.

The Examiner also reminds applicant to update the continuation data in the first line of the specification. Applicants accordingly herewith amend the specification.

The Examiner objects to the specification and rejects claims 1 and 4-6 under 35 USC §112, first paragraph, for the stated reason that the phrase "and AB heterodimer forms of platelet derived growth factor and also binds the BB homodimer with high affinity" is new matter because it cannot be found in the disclosure as filed. Applicants respectfully traverse this rejection and direct the Examiner's attention to Figures 8 and 11 and the descriptions thereof in the specification. That is, Figure 8 shows that while both ß PDGF and α PDGF receptors bound human PDGF, the pattern of competition by different PDGF isoforms distinguished the two receptors. For instance ligand AA did not bind to the ß PDGF receptor but all three ligands AA, AB and BB bound to α PDGF receptor. Figure 11 shows that both AB and AB bind α PDGF receptor at high affinity but that cells containing ß PDGF receptor had a strikingly lower affinity for PDGF AB.

Because the combined teachings of Figures 8 and 11 provide support for the recitation in claim 1, applicants respectfully request the Examiner to reconsider and withdraw this rejection.

The Examiner rejects claim 20 under 35 USC §112, first paragraph, for the alleged reason that the disclosure is enabling only for claims limited to the DNA of claim 21. Specifically, the Examiner states that in order to enable the differential hybridization cited in claim 20, the sequence information for both of the cited receptors must be instantly enabled, which is not the case. Applicants respectfully traverse this rejection and point out that, at the time of the present application's filing date, the ß PDGF receptor was known and characterized, as shown in Yarden *et al., Nature* 323: 226-232 (1986), which is hereby submitted and listed on attached Form 1449.

The Examiner has rejected claims 1-7, 16 and 18-22 under 35 USC § 112, second paragraph, as being indefinite because, according to the Examiner, claim 1 recites "sequence" as if it were a composition. In response to this rejection, applicants herewith amend claims 1 and 3 to reword the claim language, thereby eliminating the use of the term "sequence." No new matter is added with this amendment. In view of this amendment, applicants request the Examiner to withdraw this rejection.

The Examiner has rejected claims 2 and 3 under §112 for their recitation of "allelic variations." Applicants traverse this rejection but further submit that this rejection has been rendered moot in view of the amendment to claims 2 and 3 wherein the allegedly objectionable language has been removed.

CONCLUSION

In light of the above amendment and explanations, applicants assert that the specification and claims meet every requirement under § 112 and that claims 1-7, 16 and 18-22 are in condition for allowance. Early notification thereof is earnestly solicited. Examiner Marschel is invited to contact the undersigned at (202) 672-5300 to discuss any matters related to this case.

Respectfully submitted,

November 29, 1993
Date

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Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factor receptors

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The primary structure of the receptor for platelet-derived growth factor (PDGF), determined by means of cloning a cDNA that encodes the murine pre-PDGF receptor, is closely related to that of the v-kit oncogene product and the receptor for macrophage colony stimulating factor (CSF-1). Common structural features include the presence of long sequences that interrupt the tyrosine-specific protein kinase domains of each molecule. The PDGF and CSF-1 receptors also share a characteristic distribution of extracellular cysteine residues. Ubiquitin is covalently bound to the purified PDGF receptor, the human gene for which is on chromosome 5.

SEVERAL growth-stimulating peptides and their respective receptors have now been identified and characterized. Although the mechanism by which these molecules regulate proliferation is unknown, some insight into their function has come from the discovery that they are structurally and functionally similar to several of the retroviral oncogene products. Through these comparisons specific functional domains of several of the growth factor receptors can be identified. For example the receptors for insulin[1,2], macrophage colony-stimulating factor (CSF-1)[3], and epidermal growth factor[4] have, in their amino acid sequences, recognizable domains for tyrosine-specific protein kinase, an activity that is associated with both proliferation and transformation of cells[5].

One of the known growth factors, platelet-derived growth factor (PDGF), specifically stimulates the proliferation of mesenchymal cells. PDGF is similar to insulin, insulin-like growth factor I, and epidermal growth factor in stimulating tyrosine kinase activity[6–9], but it is distinctive in its ability to act early in the transition from the quiescent state to G_1 and to facilitate the subsequent actions of other growth factors[10]. It also stimulates a number of growth associated responses such as cytoskeletal rearrangement[11], turnover of phosphatidylinositol[12], and enhanced expression of a family of genes including the c-*myc* and c-*fos* proto-oncogenes[13,14]. The first step in activating the cellular responses associated with PDGF-induced mitogenesis is the interaction of PDGF with its 180 kDa cell surface receptor[15–18]. The receptor is also required for the transformation of cells by the simian sarcoma virus oncogene, v-*sis*, which encodes one of the two polypeptide chains of PDGF[19]. To gain insight into the mechanism by which the PDGF receptor mediates the responses to PDGF, we purified the receptor, determined amino acid sequences of portions of the molecule, and deduced the complete amino acid sequence of the receptor from a full length cDNA clone. In addition we determined its chromosomal position in the human genome.

Receptor purification and sequencing

The PDGF receptor was purified from BALB/c 3T3 cells in its activated form which is phosphorylated on tyrosine residues[6–9]. The purification was based on sequential affinity chromatography steps using immobilized wheat-germ agglutinin and anti-phosphotyrosine antibodies (see Fig. 1 legend), procedures that were modified from those used previously to purify analytical quantities of PDGF receptor[20]. In PDGF-stimulated cells the predominant tyrosine-phosphorylated protein that is recognized by antiphosphotyrosine antibodies is the PDGF receptor[9,20]. Thus the progress of the purification could be followed by Western blot analysis using polyclonal antiphosphotyrosine antiserum (Fig. 1*a*). By this approach the receptor protein was purified to near homogeneity as assessed by Coomassie stain (Fig. 1*b*) or by silver stain (not shown). An estimate of the extent of the purification was 4000-fold based on the intensities of the antiphosphotyrosine antibody Western blot signals. By this procedure approximately 400 μg of receptor was purified from 2,000 roller bottles of BALB/c 3T3 cells with an overall yield of 20%. Prior to sequence determination, the eluate from the antiphosphotyrosine antibody column was applied to a preparative SDS polyacrylamide gel and the receptor was eluted from the 180,000 (180K) M_r region.

Two independent receptor preparations (40 and 130 picomoles per preparation) were used for determination of the N-terminal amino-acid sequence of the receptor. In both cases there were two distinct phenyl-thio-hydantoin (PTH)-conjugated amino acids at each cycle of Edman degradation and these were present in approximately equimolar amounts. One of these sequences was identical to the amino terminal sequence of ubiquitin[22] and the other sequence (Table 1) represented the amino terminus of the PDGF receptor (see below). To determine internal sequences of the receptor, four independent trypsin digestions of the protein were performed on three separate preparations. A total of 29 peaks isolated by HPLC were analysed by amino-acid sequencing on a gas phase sequencer. Of these, 15 peaks contained homogeneous peptide species. The sequences of these peptides are presented in Table 1. One of these peptides, peptide 2, was isolated independently from each of the four tryptic digests and another, peptide 3, was isolated independently from two of the digests. Peptide 7b was found to overlap with peptide 7a, possibly the result of an abnormal tryptic cleavage after a glutamine residue. Thus the experimentally determined sequences were derived from ten independent internal peptides and from the amino terminus of the receptor.

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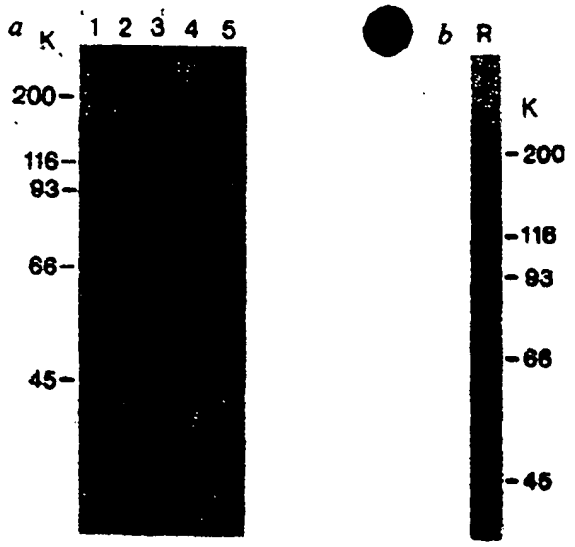

Fig. 1 *a*, Antiphosphotyrosine antibody Western blot analysis of fractions from purification steps. Aliquots from the following fractions of the purification procedure were analyzed by antiphosphotyrosine antibody Western blot: lane 1, 100 μg of cell lysate protein; lane 2, 100 μg of flow through fraction of wheat germ-agglutinin sepharose chromatography; lane 3, 1 μg of protein eluted from wheat germ agglutinin sepharose; lane 4, 1 μg of flow through fraction of antiphosphotyrosine-sepharose chromatography; lane 5, 200 ng of receptor protein specifically eluted from antiphosphotyrosine sepharose. *b*, Coomassie stain of purified PDGF receptor (lane R). An aliquot (200 ng) of the eluted receptor fraction run in lane 5 of Fig. 1*a* was analysed by Coomassie stain of a 7.5% polyacrylamide gel.

Methods. BALB/c 3T3 cells grown in roller bottles were incubated with 5 nM PDGF for 3 h at 4 °C and were then solubilized in cold Tris-buffered Triton X-100 (20 ml per bottle) containing 100 μM sodium metavanadate, 50 mM sodium chloride, 5 mM EDTA, 10 mM Tris-HCl (*p*H 7.4), 1% Triton X-100, 1 mM phenylmethylsulphonyl fluoride and 1 mg ml^{-1} bovine serum albumin. The lysates were centrifuged at 39,000 *g* for 1 h at 4 °C and supernatants were added slowly to a 30 ml column of wheat-germ agglutinin coupled to Sepharose CL-4B. After washing with 10 column volumes of the Tris-buffered Triton solution the column was eluted with 120 ml of 0.3 M *N*-acetyl glucosamine in Tris-buffered Triton. The eluates were diluted to the original volume and added to a 5 ml column of immobilized antiphosphotyrosine antibody[9,20] at a flow rate of 30 ml h^{-1} at 4 °C. The column was sequentially washed with 10 column volumes of Tris-buffered Triton without albumin, and with Tris-buffered octyl-β-D-glucoside (20 mM octyl-β-D-glucoside substitute for Triton). Phosphotyrosine-containing proteins were specifically eluted with a buffer containing 40 mM phenylphosphate, 3.3 mM Tris-HCl (*p*H 7.4), 30 mM NaCl and 20 mM octyl-β-D-glucoside. Fractions from each step were applied to 7.5% SDS-polyacrylamide gels, and transferred to a nitrocellulose filter which was sequentially incubated with antiphosphotyrosine antibody and radiolabelled goat anti-rabbit antiserum to detect the phosphotyrosine-containing proteins[21]. For amino acid sequence analysis, the purified fractions from the anti-phosphotyrosine sepharose column were concentrated, reduced, alkylated by iodacetamide treatment[55] in the presence of lithium dodecyl sulphate and were further purified by preparative SDS gel electrophoresis followed by electroelution[57]. The overall yield of purified receptor was approximately 20% (0.5–1.0 μg of receptor per roller bottle).

PDGF receptor cDNA sequence

A pool of 128 39-base oligonucleotides (Fig. 2 legend) was synthesized based on the sequence of peptide 2, and used to screen oligo(dT)-primed cDNA libraries prepared from mouse placenta and cultured NR6 mouse fibroblasts[23]. Thirty-six strongly hybridizing clones were selected: 17 from the mouse placenta library (denoted λ-MP) and 19 from the NR6 library (λ-N). To establish that we had isolated PDGF receptor cDNA sequences, two 800 bp clones (λ-N14 and λ-MP6) were sequenced using the dideoxy nucleotide chain termination procedure[24–26]. The open reading frames of the two clones overlapped for 354 nucleotides and included a sequence that coded for the peptide used to design the oligonucleotide probe. These clones hybridized to the other 34 clones. The largest clone, λ-N16, contained a 5.2 kilobase (kb) insert which was potentially large enough to encode the PDGF receptor. To confirm the identity of the clone, two additional oligonucleotide probes were synthesized based on the sequences of tryptic peptides 7a and 8 (see Fig. 2 legend). As expected, both probes strongly hybridized to the λ-N16 clone.

The complete nucleotide sequence of the cloned cDNA insert in the λ-N16 clone extends for 5,110 nucleotides, and is flanked by a poly(A) sequence at the 3′ end (Fig. 2). An open reading frame of 3,327 residues (nucleotides 106–3,432) is flanked by 105 nucleotides of 5′-untranslated sequence and 1678 nucleotides of 3′ untranslated sequence. A polyadenylation signal (ATTAAA) 17 nucleotides upstream from the poly(A) sequence is found at the 3′ end of the cDNA. The first ATG codon, found in this open reading frame at position 139, matches the consensus sequence for a translation initiation site[27]. A 17-amino-acid sequence that is homologous to the experimentally determined amino-terminal sequence of the purified PDGF-receptor protein (Table 1) begins 32 amino acids downstream from the initiation codon. Seventeen of the eighteen experimentally determined amino-terminal residues matched the sequence predicted from the cDNA (Fig. 2). Thus the leucine residue 32 amino acids from the first ATG codon is the amino terminal residue of the

Table 1 Experimentally determined peptide sequences of the PDGF receptor

Peptide	Sequence	Preparation	Recovery (pmol)
(1)	XVD(I)PLH(V)PYDHQ (E) (P)	IIb	30
(2)	(L)VEPVTDYLPGVPS	I, IIa, IIb, III	15, 10, 27, 10
(3)	LLSTLGDVE(I)AEL(H) (P) (P)	IIb, III	13, 12
(4)	(Y)VSELILV	III	8
(5)	YEI(R)	III	12
(6)	DQLVLG	I	30
(7a)	TLG(S)GARGQVVEATA (E)	III	7
(7b)	(V)VEATA(H)GL (Y)	I	21
(8)	XXPIYIITEYXFYGDLV(D)Y (G)	I	14
(9)	XXDFGLA(R)	I	30
(10)	SDHPAIL(R)	III	12
(11)	LV(I)TPP(G)P(E)FV(L)X1SSTF (N-term) (G)	A, B	50, 12

Experimentally determined peptide sequences of the PDGF receptor. Purified PDGF receptor was electroeluted from a preparative SDS polyacrylamide gel (see Fig. 1 legend), precipitated[56], and digested with trypsin. The digestion mixtures were fractionated on a reverse phase C18 column (130 T-TSK, LKB) developed with a gradient of acetonitrile in 0.1% trifluoroacetic acid. Peptide samples were sequenced by automated Edman degradation on a gas phase sequencer (Applied Biosystems) using the 02N VAC program and methanolic/HCl conversion chemistry. The PTH residues were identified and quantitated using a cyano HPLC column procedure similar to that described by Hunkapiller[57]. Total net yields were calculated at each cycle after correction for background. The recoveries based on determination of PTH-amino acids are shown. The repetitive yields were calculated by linear regression analysis through all quantitated cycles except those containing serine, threonine, histidine, and cysteine. The repetitive yields were 93–97%. The peptide sequences shown were derived from three different receptor preparations and four independent digestions. The starting amounts of PDGF receptor prior to digestion were: preparation I, 130 pmol; IIa, 200 pmol; IIb, 65 pmol; and III, 50 pmol. N-terminal sequence analysis was performed on the intact receptor (preparation A, 130 pmol, preparation B, 40 pmol). Residues are indicated by single letter amino acid codes. Residues identified but not quantitated are enclosed by parentheses. X indicates that no residue could be identified. The position of each numbered peptide in the predicted cDNA sequence is indicated by the numbers within the small squares in Fig. 2.

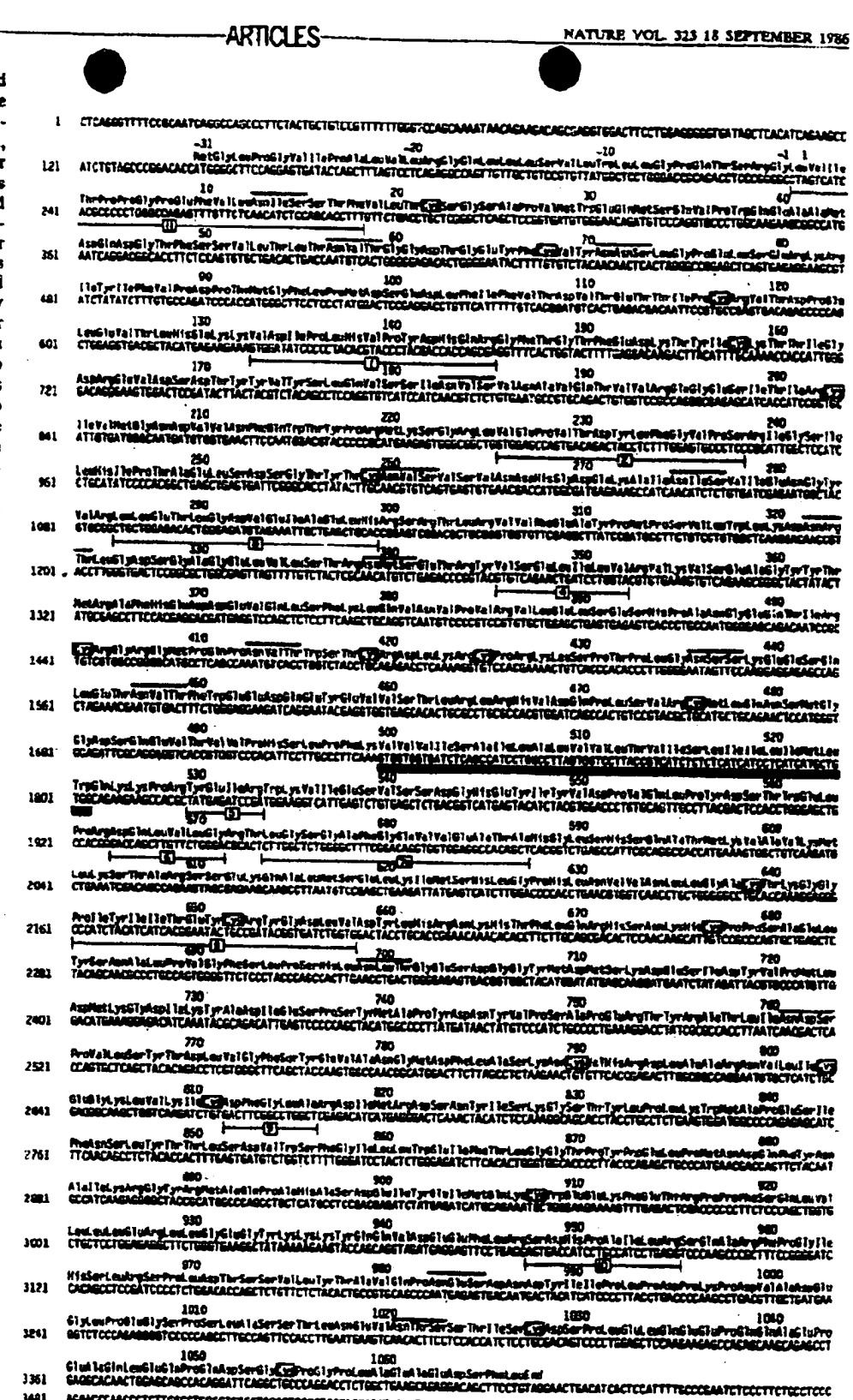

Fig. 2 Nucleotide sequence and deduced amino-acid sequence of mouse pre-PDGF receptor cDNA. Oligonucleotide probes based on peptides 2, 7a, and 8 (Table 1) of the PDGF receptor were synthesized. The DNA sequences were designed on the basis of published codon usage frequencies[59,60]. Full-length oligonucleotides (peptide 2) or overlapping partial oligonucleotides (peptides 7a and 8) from coding and noncoding strands were chemically synthesized[61]. The probe sequences for peptide 2 included all possible codon combinations for amino acids with two alternative codons. Other codons for this probe and for probes corresponding to peptides 7a and 8 were predicted on the basis of codon usage statistical data. The screening of cDNA libraries and selection of clones is described in the text. The complete sequence of the λ-N16 clone is shown in this figure. Nucleotides are numbered at the left. Amino acids are numbered over the line starting at Leu1 of the receptor sequence, and preceded by a 31 amino acid signal peptide. Peptide sequences derived from purified receptor preparations are underlined and numbered according to Table 1. Potential sites of N-linked glycosylation are overlined. The putative single transmembrane region is demarcated by a black bar. The ATTAAA box close to the polyadenylated 3′ end of the cDNA is underlined.

Methods. Synthetic probes were radiolabelled by 5′-end phosphorylation[62] using T4 polynucleotide kinase (PL Biochemicals) and [γ-^{32}P]ATP (Amersham > 5,000 Ci mmol^{-1}). Synthetic probes corresponding to peptides 7a and 8 were further labelled by filling in the complementary sequence using *Escherichia coli* DNA polymerase I (Klenow fragment, Boehringer-Mannheim) and [α-^{32}P]dCTP and [α-^{32}P]dATP (> 5,000 Ci mmol^{-1}, Amersham). The resulting radiolabelled oligonucleotides were separated from radionucleotides by gel filtration through Sepharose G-50 (Pharmacia). This procedure yielded specific activities of $3–6 \times 10^8$ c.p.m. per μg. Total poly(A)-containing RNA from cultured mouse NR6 cells and mouse placenta was prepared by the guanidine thiocyanate-LiCl method[64] followed by passages through an oligo(dT)-cellulose column[65]. A clone library was constructed as described[66] using the λgt10 vector system (Vector) and was screened by hybridization to the radiolabelled oligonucleotide corresponding to tryptic peptide 2. Hybridization to the λ-phage containing nitrocellulose filters was carried out at 40 °C for 16 h under low stringency conditions; 20% formamide, 5× SSC (1× SSC = 150 mM NaCl, 15 mM Na_3 citrate). Nucleotide sequence analysis was carried out by subcloning of restriction fragments of λ-N16 recombinant phage clone into M13-based cloning vectors followed by primed DNA synthesis on single-stranded DNA templates in the presence of dideoxynucleotide triphosphates[24,26].

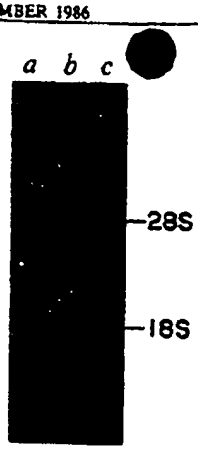

Fig. 3 Identification of PDGF receptor mRNA by Northern blot analysis. Total poly(A)-containing RNA was isolated from cultured human skin fibroblasts (*a*), A431 cells (*b*), and NR6 mouse fibroblasts (*c*) using the guanidine-monothiocyanate-LiCl method of cell homogenization[64] followed by oligo(dT)cellulose chromatography[65]. The RNA from NR6 cells was re-chromatographed on oligo(dT) cellulose. RNA (4 μg) was heated for 10 min at 65 °C in the presence of formamide and formaldehyde and then subjected to electrophoresis on a 1.2% agarose gel which contained formaldehyde[63]. After transfer to nitrocellulose the samples were hybridized at 42 °C with nick-translated[66] λ-N16 cDNA insert (5,134 base pairs, Fig. 2) in 50% formamide, 5×SSC and 50 μg ml^{-1} denatured salmon sperm DNA. The filters were then washed at 50 °C with 0.2×SSC, 0.1% SFS. Exposure was for 5 days at −60 °C using an intensifying screen. Calf thymus ribosomal RNA was used as a size standard. (4,718 bp and 1,874 bp).

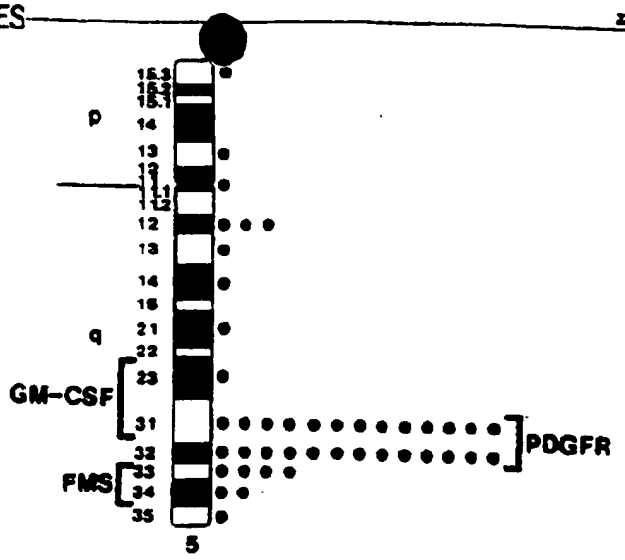

Fig. 4 Autoradiographic silver grain distribution along chromosome 5 after *in situ* hybridization with λ-N16 cDNA insert (ideogram from ISCN 1985)[67]. The methods have been described[68]. Brackets on the left mark the published localizations of the gene for GM-CSF[35,36] and *fms*[36,37]. Specific labelling at bands 5q31→5q32 indicates position of PDGF receptor locus.

mature PDGF receptor. The amino acid residues between the ATG codon and the amino terminus of the mature protein are hydrophobic and likely represent the signal peptide sequence necessary for transport of the nascent pre-PDGF receptor precursor into the lumen of the endoplasmic reticulum[28–30].

The experimentally determined amino acid sequences of the ten tryptic peptides could be located in the predicted amino acid sequence of the open reading frame of the cDNA insert of clone λ-N16. The peptide sequences matched the cDNA-derived amino acid sequence for all 120 experimentally determined amino acids except for residue 12 of peptide 8 which was identified to be phenylalanine but predicted from the cDNA to be arginine. The cDNA sequence also predicted the tryptic cleavage sites at the amino termini of the peptides. Taken together these findings show that the cloned cDNA insert in the λ-N16 clone represents the coding sequence of the PDGF receptor mRNA.

In addition to the signal sequence, the deduced 1098 amino acid polypeptide sequence contains features characteristic of cell surface glycoprotein receptors. These include a stretch of 25 hydrophobic amino acids characteristic of a membrane-spanning domain[31] (underlined by a black bar in Fig. 2), which is flanked at its carboxyl side by a basic sequence typical for the junction between the membrane and cytoplasmic domains of cell surface receptors[1–4,32–33]. Fifteen consensus sequences for asparagine-linked glycosylation (Asn-X-Ser/Thr) are distributed with a preference for the amino-terminal and presumably extracellular half of the sequence (11 potential sites). Nineteen cysteine residues are found distributed over the pre-PDGF-receptor sequence (boxed type in Fig. 2).

Single receptor mRNA

To determine the size of the PDGF receptor mRNA, Northern blot hybridization experiments were carried out using the cDNA insert of λ-N16 clone as hybridization probe. Figure 3 shows that a single band is visualized in cytoplasmic poly(A)$^+$ RNA of NR6 mouse fibroblasts, in contrast to multiple mRNA species reported for other receptor tyrosine kinases such as EGF and insulin receptors[1,2,4]. This single mRNA species is approximately 5.3 kb in size, indicating that our cloned cDNA represents most if not all the messenger RNA encoding the mouse pre-PDGF receptor.

In a survey of mouse tissues and cell lines (data not shown) relatively high levels of the 5.3 kb mRNA species were found in kidney and placenta, and lower levels were detected in brain and testes. Likewise, all 3T3 mouse fibroblasts tested contained a similar band. However, 3T3-L1 adipocytes contained only 20% as much receptor message as 3T3-L1 fibroblasts, and NR6 cells contained only 30–40% as much as BALB/c 3T3 fibroblasts. High levels of PDGF receptor mRNA were also found in PDGF-responsive human foreskin fibroblasts, but not in human epidermal carcinoma A431 cells which are known to be devoid of PDGF receptors[34] (Fig. 3). Human term placenta and a 16-day mouse placenta contained elevated levels (2–4-fold) of PDGF-receptor mRNA when compared with mRNAs from earlier stage of pregnancy (20th-week human placenta and 10th-day mouse placenta).

Chromosomal location of PDGF receptor

The chromosomal location of the human PDGF receptor gene was determined by *in situ* hybridization of the 5.2-kb mouse PDGF receptor cDNA fragment (λ-N16) to chromosome preparations. Of 120 cells analysed, 26 (22%) had silver grains on bands (5q31→5q32) accounted for 10% (26/259) of total grains scored. No other chromosomal site was labelled above background. This location of the PDGF receptor gene is between the loci of the granulocyte-macrophage colony-stimulating factor (GM-CSF) gene (5q23→5q31)[35,36] and the *c-fms* gene (5q33→5q34)[36,37].

Southern blot analysis of DNA prepared from ten human × Chinese hamster somatic cell hybrids confirmed the localization of the PDGF receptor gene to chromsome 5. The hybrids carrying human chromosome 5 all contained the 7.3-kb human-specific *Hin*dIII fragment and a 2.6-kb human fragment visible after longer exposure (not shown). Three fragments, 17 kb, 9.3 kb and 2.2 kb, were present in Chinese hamster DNA and in all hybrids. Chromosome 5 was the only human chromosome that showed perfect concordance with human PDGF receptor sequences. Every other human chromosome could be ruled out by at least two discordant hybrids.

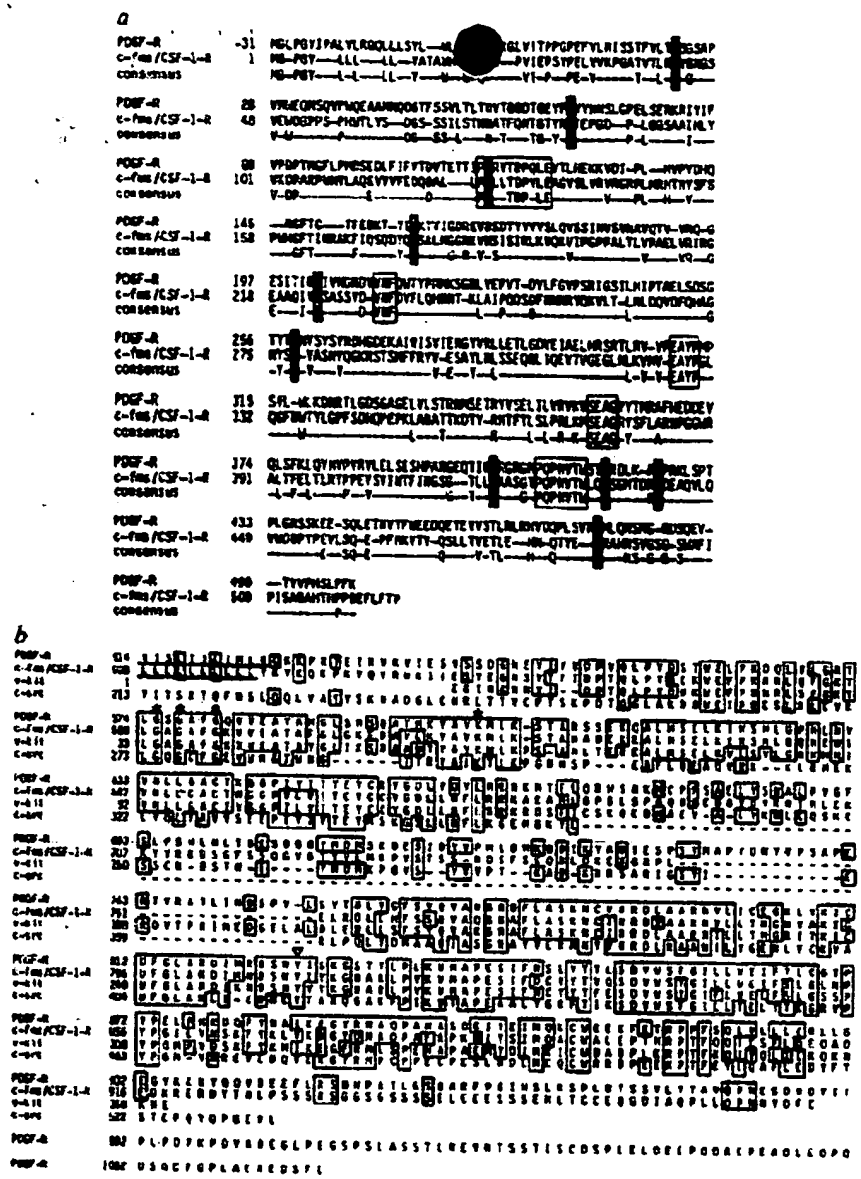

Fig. 5 *a*, Comparison of the amino acid sequence of the extracellular domain of the pre-PDGF receptor with the sequence of c-*fms*/CSF-1 receptor[3]. The predicted extracellular amino-terminal halves of both proteins are aligned and gaps are introduced for optimal alignment. The putative signal sequences are included in the comparison. Clusters of conserved amino acids between the proteins are boxed and all cysteine residues are shaded. Amino acid numbers are given preceding each line of sequence. Leu1 of PDGF receptor is the experimentally determined amino-terminus of the protein. As the precise location of the amino-terminus of the c-*fms* protein is still unknown, the numbering of c-*fms* starts at the initiation methionine[3]. *b*, Comparison between the cytoplasmic domain of PDGF receptor and other tyrosine kinases. The predicted amino acid sequence of PDGF receptor is compared with that of the closely related genes, c-*fms*/CSF-1 receptor[3] and v-*kit*[39]. The sequence of the c-*src* gene[40] is also included for comparison. Only the cytoplasmic sequences of PDGF receptor and c-*fms*/CSF-1 receptor and portions of the respective transmembrane domains (underlined) are aligned. Residues which are identical in PDGF receptor and other genes are boxed. Gaps, shown by hyphens, were introduced for optimal alignment. Asterisks demarcate residues thought to be involved in nucleotide binding. The open arrow indicates the conserved tyrosine acceptor site homologous to Tyr416 of pp60$^{v\text{-}src}$[40].

Discussion

The 180 K protein which we have identified as the PDGF receptor has tyrosine kinase enzyme activity[20] and is the same size and has a similar isoelectric point as the PDGF receptor identified by ^{125}I-PDGF cross-linking experiments[9,18]. Its overall structure, deduced from the cDNA sequence, is consistent with that of a receptor and includes a transmembrane domain, a tyrosine kinase domain, and potential sites for N-linked glycosylation in the extracellular domain. In previous studies we showed that high affinity ^{125}I-PDGF binding copurifies (10,000-fold) with this protein[20]. The protein and its message are present in cells known to respond to PDGF and both are absent from cells that are unresponsive (Fig. 3). The yield of PTH-amino acids determined in the sequence analyses of the amino terminus and the internal peptides, suggests that the sequences are, in fact, from this protein and not from a minor contaminant (see Table 1).

The protein product predicted by the cDNA clone would have an apparent M_r of 120 K after removal of the signal sequence. In recent experiments we have shown that glycosidase treatment of biosynthetically labelled PDGF receptor removes sialic acid and N-linked oligosaccharide moieties and causes a 30–40 K decrement in apparent molecular weight (T.D. *et al.*, in preparation). Other post-translational modifications such as addition of O-linked oligosaccharide, ubiquitin, or phosphate groups may contribute to the remainder of the difference between the apparent weight and the predicted weight.

Inspection of the predicted primary structure of the PDGF receptor reveals structural features common to other cell-surface receptors for growth factors (Fig. 5). These include a single stretch of predominantly hydrophobic amino acids (residues 500–524) capable of serving as a transmembrane anchor sequence, an amino-terminal extracellular domain (residues 1–499) that should contain the ligand binding region (Fig. 5*a*) and an intracellular domain (residues 525–1,067) carrying the enzymatic phosphotransferase activity (Fig. 5*b*).

The PDGF receptor tyrosine kinase domain, which can be identified by homologies with the transforming proteins of the *src* family of tyrosine kinases[1–5], is divided into two distinct regions (residues 572–662 and 767–919) separated by a 104-amino-acid sequence (residues 663–766) that is unrelated to known tyrosine kinase sequences (Fig. 5*b*). Inserted sequences that interrupt kinase domains at analogous locations of the v-*fms*[38], c-*fms*[3] and v-*kit*[39] genes have been recently identified. Notably the insertion of v-*kit* shows significant homology with the insertion of PDGF receptor (24 identities out of the 79 residues of the insertion of v-*kit*) as compared with only six identities between the somewhat shorter insertion of c-*fms*/CSF-1 receptor and PDGF-receptor. All three insertion sequences

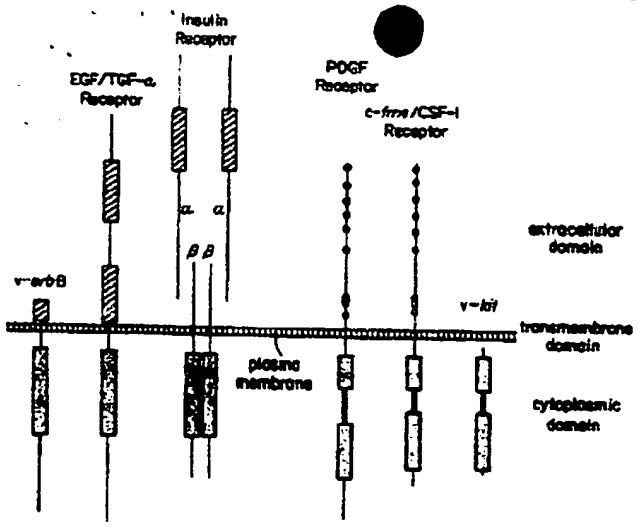

Fig. 6 Topological comparison of the two groups of tyrosine-kinase receptors and their oncogenic variants. All proteins shown are oriented so that their carboxy-termini are in the cytoplasm. Hatched boxes indicate regions that are rich in cysteine residues. Protein domains that share high homology with other tyrosine-kinases of the *src* gene family are shown by stippled boxes. Solid lines show stretches of sequences inserted within the tyrosine-kinase regions of PDGF receptor, c-*fms*, and v-*kit*. The distribution of cysteine residues in the extracellular domains of PDGF-receptor and c-*fms*/CSF-1 receptor is indicated by closed circles, whereas hatched boxes show cysteine-rich repeat domains found in the extracellular ligand-binding regions of EGF receptor, insulin receptor, and HER2/*neu*.

are relatively hydrophilic. Considering its position, the inserted sequence may play a role in cytoplasmic substrate binding or in modulation of kinase activity.

The expected tyrosine kinase landmarks are found in the split PDGF receptor kinase domains. The amino-terminal kinase domain (residues 572–662) contains residues which are thought to be involved in formation of the nucleotide binding site including Lys_{602} and a Gly-X-Gly-X-X-Gly sequence[40]. The sequence of this region (residues 572–662) is highly homologous with members of the *src* family of kinases, the closest match being c-*fms* (68% homology) which is thought to be the receptor for CSF-1[41]. The second kinase region (residue 767–919) contains most of the conserved kinase sequences, including the homologous sequences surrounding the major site of autophosphorylation (Tyr 416) of $pp60^{v\text{-}src}$ [42].

Other portions of the PDGF receptor sequence, in addition to the kinase domains, show striking homology to the c-*fms*/CSF-1-receptor sequence. The extracellular domains have a very similar pattern of cysteine distribution (Fig. 5*a*) and have a 30% sequence homology which is due mainly to short conserved sequences and conservation of all of the cysteine residues. Significant homology (34%) is also found in the stretch of 47 amino acids (residues 525–571) between the transmembrane region and the kinase domain (Fig. 5*b*). The two kinase domains of the PDGF receptor are the most homologous to c-*fms*/CSF-1-receptor sequences (72% and 64% respectively). The sequence that interrupts the kinase domain (residues 663–766) and the 148-amino-acid carboxy-terminal region of the PDGF receptor are the least homologous to the corresponding c-*fms*/CSF-1-receptor regions (8% and 13% respectively) (Fig. 5*a*). Taken together these findings show that the PDGF receptor and the c-*fms*/CSF-1-receptor are closely related, both in general structural features (cysteine distribution, location of kinase domains and presence of insert regions) and in specific sequences. The intracellular domain of the PDGF receptor is also closely related to the sequence of the v-*kit* transforming gene of HZ4 feline retrovirus[39] (Fig. 5*b*). This homology is apparent in both the tyrosine kinase sequences (63% homology) and in the short region of v-*kit* which is between the *gag* sequences of the viral fusion protein and the amino terminus of the kinase domain (53% homology). Thus the normal cellular homologue of the v-*kit* oncogene may encode a receptor that is closely related to the receptor for PDGF and CSF-1.

Based on the structural features of the PDGF receptor and on the presented sequence homologies (Fig. 5) it is possible to divide the known receptor tyrosine kinases into two subgroups which are schematically depicted in Fig. 6. The first subgroup, which includes the PDGF receptor, c-*fms*/CSF-1-receptor[3], and the v-*kit* protein[39] (and its putative normal cellular homologue), is characterized by kinase domains with long inserted sequences, and in the case of the PDGF receptor and c-*fms*/CSF-1-receptor, a similar pattern of cysteine distribution in the extracellular domain. The other subgroup includes the EGF receptor[4], the insulin receptor[1,2] as well as HER2/*neu* oncogene[43–45], which is thought to be a receptor of an as yet unidentified ligand. Their common structural elements include partially homologous extracellular domains with characteristic cysteine-rich regions and uninterrupted intracellular kinase domains (Fig. 6). It is of interest that unlike insulin and EGF, both PDGF[46] and CSF-1[47] are disulphide-linked dimeric molecules. Whether the structural similarity of these ligands is related to the structural similarity of the receptors remains to be determined.

A striking finding in the experimentally determined PDGF receptor sequence was the presence of two amino terminal sequences. One of these was the predicted amino terminus of the processed protein encoded by the cDNA clone, and the other was the sequence of the 8.5 K peptide, ubiquitin. The most likely explanation of these findings is that ubiquitin is covalently bound to the PDGF receptor, presumably through an amide bond between the carboxyl-terminal amino acid of ubiquitin and an ξ-amino group of a lysine residue of the receptor. The role of this modification of the receptor is not known. Ubiquitination of other proteins appears to pay a role in protein degradation[48] and specific ubiquitination of histones appears to be involved in transcriptional control[49,50]. Recently Siegelman *et al.*[51] have reported that ubiquitin is conjugated to the lymphocyte homing receptor, possibly in the extracellular domain. Thus there may be other surface molecules, possibly some receptors, that are modified by ubiquitination. It is possible that this post-translational modification plays a role in signal transduction or receptor processing.

The human PDGF receptor gene is located near the gene for the c-*fms*/CSF-1 receptor protein. Acquired partial deletions of this region of chromosome 5 are found in a number of haematologic disorders[52–55]. Since the chromosomal breakpoints in these disorders cluster in bands 5q13→5q15 and 5q31→5q34[52,55], it is possible that in at least some of these patients there are alterations in the PDGF receptor gene. Whether these alterations are functionally important remains to be determined.

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Infrared point sources aligned with the SgrA* non-thermal radio source

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Recent observations[1,2] have revealed point sources at wavelengths 0.7–1.0 μm and 1–5 μm, which are approximately aligned with the compact non-thermal radio source SgrA* in the galactic centre. The assembled 0.7–5 μm data indicate that the two point sources are at the same position and have spectral continuity; they are thus probably the same object. Here we discuss alternative interpretations of this infrared point source. If it is a foreground star, it must be a hot star surrounded by a circumstellar dust cloud. If it is an object at the galactic centre, an unorthodox extinction curve is required to derive an intrinsically hot black-body spectral distribution, $F_\nu \propto \nu^2$, over the 0.7–3.5 μm wavelength range. Under these circumstances the infrared emission may be the Rayleigh–Jeans tail of a hot star or star cluster, or thermal accretion disk with temperature $\approx 35 \times 10^3$ K.

The physical relationship between the infrared point sources[1,2] and the SgrA* source[3,4] is unclear, in that the former object or objects are possibly foreground sources which are not actually in the region of the galactic centre[5]. The observed flux levels from the results of Biretta *et al.*[3] and Forrest *et al.*[2] over the frequency range $13.9 < \log \nu < 14.65$ (wavelength range $0.7 < \lambda < 3.75$ μm) are plotted in Fig. 1. We have used the conversion from AB (defined in ref. 6) to F_ν (erg cm^{-2} s^{-1} Hz^{-1}) given in ref. 6, $AB = -2.5 \log F_\nu - 48.60$ (a misprint in ref. 6 gives +48.60) for the $0.7 < \lambda < 1.0$ μm data. In addition to the alignment in direction of the Biretta *et al.*[3] object A (also called CCD2; refs 7, 8) and the object designated IRS16NW by Forrest *et al.*[2], we note that there is spectral-flux continuity as well, before corrections for reddening are applied. The directional alignment within 0.2 arcs and spectral-flux continuity lead us to conclude that object A and IRS16NW are the same object. We adopt this conclusion, but we do not assume that this infrared source has any relationship to SgrA* (see also ref. 9).

The difficulty of interpreting the 0.7–5 μm observations of the infrared point source (object A+IRS 16NW) arises in attempting to correct the observed flux levels as a function of frequency for extinction and reddening due to intervening dust. There are two initial possibilities to be investigated: the infrared source (A+16NW) may be a foreground star[5], or it may lie in the vicinity of the galactic centre. We illustrate various possible intrinsic spectral-flux distributions of the infrared point source in Fig. 1, which result from applying a standard extinction law as a function of wavelength for assumed V-band extinctions ($\lambda = 0.55$ μm), $A_V = 10$, 20 and 30 mag. We have adopted the extinction curve of Becklin *et al.*[10] plus van de Hulst no. 15 (ref. 11) in these corrections. The values of A_V represent the range of visual extinction that might be expected for a foreground star in the direction of the galactic centre, up to those values expected for sources in the galactic centre ($A_V = 30$ mag; ref. 12).

The extinction corrections shown in Fig. 1 illustrate the difficulty of interpreting the infrared point source observations over the entire $0.7 < \lambda < 3.5$ μm spectral interval. Spectral distributions resulting from the corrections for $A_V = 20$ and 30 mag are exceedingly steep between 0.7 and 1.0 μm. We agree with Biretta *et al.*[3] that there seems to be no natural physical explanation for such a steep spectrum at these wavelengths. It is this result that led Biretta *et al.*[3] to suggest that their object A is a foreground star.

The recent results of Forrest *et al.*[2], however, complicate the interpretation of the infrared point source as a simple, early-type star. Our de-reddening calculations for $A_V = 10$ mag show that this star would have a large infrared excess at wavelengths beyond $\lambda = 1$ μm, presumably attributed to a circumstellar dust shell. This would be the natural interpretation of observations such as those shown in Fig. 1 (see ref. 13 and refs therein). We can determine, approximately, the nature of the hypothesized